Survey of *Fusarium sambucinum* (*Gibberella pulicaris*) for Mating Type, Trichothecene Production, and Other Selected Traits

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ABSTRACT

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The bisexual heterothallic ascomycete *Gibberella pulicaris* (anamorph = *Fusarium sambucinum*) is an important trichothecene-producing plant pathogen that has been used to study the genetics of trichothecene synthesis and pathogenicity by analysis of naturally occurring variants. To obtain additional useful natural variants, 53 strains, identified as *F. sambucinum*, were tested for their ability to make trichothecenes and to form fertile crosses. Eight new fertile strains were identified. These eight strains, plus 40 of the nonfertile strains, made detectable trichothecenes in liquid shake culture. In all cases, 4,15-diacetoxyscirpenol (DAS) was the primary trichothecene produced. However, variations in the level of trichothecenes produced by the eight new fertile strains indicated that they may provide unique alleles for trichothecene production genes. Comparison of the data from this study to that previously published for 20 other *G. pulicaris* strains revealed a high correlation between trichothecene production, sexual fertility, and the original isolation of the strain from a diseased plant. These correlations suggest that trichothecene production may be involved in both the pathogenicity and fertility of *G. pulicaris*.

Additional keyword: mycotoxin.

Gibberella pulicaris (Fr.:Fr.) Sacc. (anamorph = *Fusarium sambucinum* Fuckel) is a cosmopolitan soil saprophyte, a trichothecene-producer, and a plant pathogen that causes cankers on woody trees, root and seedling rots of cereals, and storage rots of fruits and potatoes (7,9,24). The trichothecene production capability of *G. pulicaris* and related fungi is an important agricultural concern. In crops, the presence of trichothecenes, which are potent inhibitors of eukaryotic protein synthesis (26), poses serious health problems for both animals and humans (24,30). To eliminate trichothecenes from food crops, it will be necessary to prevent trichothecene toxin production and/or fungal contamination. A better understanding of the genetic and biochemical mechanisms involved in the pathogenicity and mycotoxigenicity of trichothecene-producing fungi can help accomplish these goals.

G. pulicaris is well-suited for investigating trichothecene production and plant-fungal interactions at the genetic, biochemical, and molecular biological levels (3,10). This heterothallic ascomycete is amenable to tetrad analysis (5), mutant selection (3), transformation (27), and a rapid, simple pathogenicity assay (11). Genes for trichothecene production, as well as several genes for the pathogenicity of this fungus on potato tubers, have been identified by crosses between natural variants (5,10,11). In addition, plant-

fungal interactions that involve both trichothecenes and the phytoalexins, rishitin, lubimin, and xanthotoxin, have been studied at the biochemical level (11,12,14,17).

Natural variants provided the genetic material used to identify at least three *G. pulicaris* genes involved in trichothecene production (3,10). Other trichothecene toxin (*Tox*) genes exist (2,4,21,22), and variants for them may also be present in the natural population. The primary objective of the present study was to characterize additional *F. sambucinum* strains for their sexual compatibility and their ability to make trichothecenes. We report here the identification of eight new fertile strains, all of which produce trichothecene toxins, and present data consistent with a single bipolar mating population for *G. pulicaris*.

MATERIALS AND METHODS

Strains, media, and culture conditions. Information regarding the strains used in this study is provided in Table 1. The strains are grouped in three categories (mating type 1 strains, mating type 2 strains, and nonfertile strains), and they are arranged numerically, within each catagory, by strain number. On receipt, each strain was reisolated from a single conidium. Conditions for growth and storage of isolates on V8 juice agar medium were as previously reported (5,10). Liquid shake cultures were grown in YEPD-5G medium (0.1% yeast extract, 0.1% peptone, and 5.0% glucose) (10,31). Each culture (25 ml of medium in 50-ml Erlenmeyer flasks) was inoculated to a final concentration of 10^4–10^5 conidia per milliliter. The inoculum was prepared by washing conidia from the surface to 5- to 14-day-old cultures grown on V8 juice agar plates. After 7 days incubation at 28 C and 180–200 rpm, the liquid cultures were stored at −20 C. Before freezing, mycelia from 5-ml fractions were collected to monitor growth as measured by dry weight (10). V8 juice agar and Martin's medium (1.0% glucose, 0.5% peptone, 0.1% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$ and 2.2% agar) were used for drug sensitivity testing as described in the Results section.

Cross conditions. As a bisexual heterothallic ascomycete, *G. pulicaris* displays two mating types. In this paper, we used Mat-1 and Mat-2 as the phenotypic designation for the two mating types; *MAT*1 denotes the mating type locus, and *MAT*1-1 and *MAT*1-2 denote the two mating type alleles. Strains may also be either male (Mln^+ or m), female (Fmn^+ or f), both or neither. Femaleness is scored by the ability of a strain to form protoperithecia; a fertile female is also able to function as the recipient strain in genetic crosses. Maleness is determined by the ability of a strain to function as the donor strain (10).

TABLE 1. Source and characteristics of *F. sambucinum* strains used in this study

Item	Strain number: Original[a]	Strain number: Single spore[b]	Source	Geographical location[c]	Origin[c]	Mating type[d]	Sex[e]	DAS μg/ml[f]
1.	R-583[g]	A-26567	P. Nelson	England	Knotweed	1	mf	3
2.	R-2633[h]	NRRL-13701	P. Nelson	USA, ID	Potato	1	m	298
3.	R-5390[i]	NRRL13703	P. Nelson	Iran	Potato	1	mf	40
4.	R-5920	A-26583	P. Nelson	Australia, NSW	Pine	1	m	tr
5.	R-5928	A-26584	P. Nelson	Australia, NSW	Soil	1	m	tr
6.	R-6112[i]	A-26585	P. Nelson	Australia	Soil	1	m	21
7.	R-6380[i]	NRRL-13708	P. Nelson	Germany	Potato	1	mf	74
8.	KF-702		J. Chelkowski	Poland	Potato	1	mf	42
9.	KF-710		J. Chelkowski	Poland	Potato	1	mf	51
10.	KF-728[h]	NRRL-13704	P. Golinski	Poland	Potato	1	m	136
11.	KF-735[h]	NRRL-13705	P. Golinski	Poland	Potato	1	m	112
12.	DAOM-192963[h]	NRRL-13503	G. Neish	Canada, P.E.I.	Potato	1	mf	93
13.	DAOM-192966[h]	NRRL-13504	G. Neish	Canada, P.E.I.	Potato	1	mf	53
14.	DAOM-196035[h]	NRRL-13709	G. Neish	Canada, N.B.	Potato	1	mf	14
15.	A-27952[h]	NRRL-13707	S. Leach	USA, ME	Potato	1	mf	104[j]
16.	A-27960[h]	NRRL-13711	A. Desjardins	USA, CO	Potato	1	m	5
17.	None[g]	NRRL-13500	R. Caldwell	USA, WI	Potato	1	mf	75
18.	None	A-27966	A. Bonnen	Unknown	Unknown	1	m	98
19.	None	NRRL-13495	P. Nelson	Unknown	Unknown	1	m	114
20.	R-2882[h]	NRRL-13710	P. Nelson	Australia	Potato	2	mf	12
21.	R-5389[h]	NRRL-13706	P. Nelson	Iran	Potato	2	m	80
22.	R-5455[i]	A-26576	P. Nelson	USA, MN	Corn	2	mf	1
23.	R-6354[i]	A-26586	P. Nelson	Canada	Corn	2	m	3
24.	R-7843[g]	A-27980	P. Nelson	Chile	Carnation	2	m	2[j]
25.	A-27937	A-27961	M. Crawford	Unknown	Unknown	2	m	2
26.	A-27939[h]	NRRL-13700	A. Murphy	Canada, N.B.	Potato	2	m	204
27.	A-27940	A-27964	A. Murphy	Canada, N.B.	Potato	2	mf	32[j]
28.	R-110	A-26566	P. Nelson	USA, MN	Pine	Nonfertile	f[e]	1
29.	R-2155	A-26568	P. Nelson	Australia	Unknown	Nonfertile		tr
30.	R-3032	A-27969	P. Nelson	Australia, NSW	Grass	Nonfertile		tr
31.	R-3084	A-27970	P. Nelson	Australia, NSW	Grass	Nonfertile		0[j]
32.	R-3245	A-27971	P. Nelson	Australia, NSW	Grass	Nonfertile		1
33.	R-3248	A-27972	P. Nelson	Australia, NSW	Grass	Nonfertile		tr
34.	R-4170	A-27973	P. Nelson	Australia, NSW	Grass	Nonfertile		tr
35.	R-4187	A-27974	P. Nelson	Australia, NSW	Grass	Nonfertile		tr

(continued on next page)

[a] All R numbers are strains from the *Fusarium* Research Center. Strains referenced in footnotes g, h, and i have been previously tested for fertility and trichothecene production.
[b] A and NRRL numbers are strains from the Northern Regional Research Center collection.
[c] Data from investigators who supplied cultures.
[d] Mating type determined by crossing to mating type tester strains R-6380 (*MAT*1-1) and R-5455 (*MAT*1-2).
[e] Femaleness determined by the ability of a strain to form protoperithecia; R-110 made protoperithecia but it did not function as a recipient.
[f] Trichothecenes analyzed in 7-day liquid shake cultures by gas chromatography analysis as described in Materials and Methods; (tr [trace] quantities are ≤1 μg/ml 4,15 diacetoxyscirpenol).
[g] Data on mating type are from (5).
[h] Data on mating type and sex were previously published (13), but entries 12, 15, and 17 were incorrectly reported as male only.
[i] Data on mating type and sex are from (10).
[j] Trichothecene production tested by gas chromatography-mass spectral analysis.

In this study, all strains were crossed as both a female (recipient) and a male (donor) to two bisexual tester strains, R-6380 (Mat-1, Mln^+, Fmn^+, Red^-) and R-5455 (Mat-2, Mln^+, Fmn^+, Red^+), of opposite mating type according to previously described conditions (5,10). Strains that produce a red pigment on potato-dextrose agar are designated Red^+; those that do not are Red^- (10). In some cases, crosses were repeated up to five times before a fertile cross was obtained. Random ascospore and tetrad isolations were performed as previously described (5,10). In *G. pulicaris*, the four meiotic products undergo one mitotic division to yield asci that contain eight spores that are four sets of twins. Accordingly, asci from which either seven or eight spores are isolated constitute complete tetrads. In this study, the segregation ratios for tetrad analysis are based on the four meiotic products from each tetrad rather than on all the individual spores.

Trichothecene analysis. The ability of strains to produce trichothecenes in liquid shake culture was examined by gas chromatography (GC). Culture extracts were prepared essentially as described by Desjardins and Beremand (10). The trichothecenes were analyzed as either trimethylsilyl (TMS) derivatives or as heptafluorobutyrate (HFB) derivatives. In both cases, portions (5–400 μl) of the culture extracts were evaporated to dryness under liquid nitrogen. The evaporated samples were then either reacted with 100 μl of Tris-Sil/TBT (Pierce Chemical Co., Rockford, IL.) at 80 C for 1 h and brought to 1 ml with hexane, or they were treated with N-heptafluorobutyrylimidazole (Pierce Chemical Co.) as previously described (1). 4,15-Diacetoxyscirpenol (DAS) concentrations were determined by comparison to a standard curve generated for each analysis by chromatography of known concentrations of commercially purified DAS (Sigma Chemical Co., St. Louis, MO.).

The TMS derivatives were analyzed on fused silica capillary columns by flame ionization detection. Samples (2 μl) were injected in the splitless mode into either a Spectra Physics 7100 GC fitted with a DB-1 coated (0.25 μm) capillary column (30 m × 0.25 mm; J&W Scientific, Folsom, CA) or a Hewlett Packard 5890 GC fitted with an HP-1 coated (2.65 μm) capillary column (5 m × 0.5 mm; Hewlett Packard, Palo Alto, CA). Both GCs were operated under equivalent conditions and quantitatively identical results were obtained. Samples were introduced at an initial oven temperature of 120 C and the column was immediately heated at 15 C/min to 210 C; after 1 min at 210 C, the column was heated at 5 C/min to 260 C and then held at 260 C for 10 min. (6,21).

The HFB derivatives were analyzed by electron capture detection on a Spectra Physics 7100 GC according to previously described conditions (1). Undiluted extract preparations were assayed first; if the trichothecene levels were high, a dilution of the sample was then analyzed.

As listed in Table 1, samples 24, 25, 27, 30–39, and 51–68 were analyzed as HFB derivatives; all other samples were assayed as TMS derivatives. Both methods are quantitatively equivalent, but the HFB method is more sensitive and allows detection of smaller quantities of the trichothecenes. Finally, as indicated in Table 1, the presence or absence of DAS in eight of the cultures was confirmed by GC-mass spectral analysis of the culture extracts using previously described methods (13).

Scoring other cultural traits. Drug sensitivity was determined by comparing growth rates in the presence and absence of test compounds. Each compound was incorporated into V8 juice agar or Martin's medium (polymyxin and enniatin B only) from dimethyl sulfoxide (DMSO) stock solutions; the final DMSO concentration was 1.0% v/v. Small plates (3 cm diameter) containing 1 ml of medium were inoculated on one edge with a myce-

TABLE 1. *(continued from preceding page)*

Item	Strain number		Source	Geographical location[c]	Origin[c]	Mating type[d]	Sex[e]	DAS μg/ml[f]
	Original[a]	Single spore[b]						
36.	R-4263	A-27975	P. Nelson	Australia, NSW	Grass	Nonfertile		tr
37.	R-4268	A-27976	P. Nelson	Australia, NSW	Grass	Nonfertile		tr
38.	R-4272	A-27977	P. Nelson	Australia, NSW	Grass	Nonfertile		tr
39.	R-4273	A-27978	P. Nelson	Australia, NSW	Grass	Nonfertile		tr
40.	R-5185	A-26571	P. Nelson	Unknown	Unknown	Nonfertile		tr
41.	R-5214	A-26572	P. Nelson	Great Britain	Soil	Nonfertile		3
42.	R-5344	A-26573	P. Nelson	Australia, NSW	Pine	Nonfertile		3
43.	R-5683	A-26577	P. Nelson	Australia, NSW	Soil (pine nursery)	Nonfertile		tr
44.	R-5684	A-26578	P. Nelson	Australia, NSW	Soil (pine nursery)	Nonfertile		tr
45.	R-5690	A-26579	P. Nelson	Australia, NSW	Soil (pine nursery)	Nonfertile		tr
46.	R-5749	A-26580	P. Nelson	Australia	Soil (pine nursery)	Nonfertile		tr
47.	R-5753	A-26581	P. Nelson	Australia, NSW	Soil (pine nursery)	Nonfertile		tr
48.	R-5867	A-26582	P. Nelson	Australia	Soil (pine nursery)	Nonfertile		tr
49.	R-7570	A-26587	P. Nelson	South Africa	Soil debris	Nonfertile		338
50.	R-7715	A-26588	P. Nelson	Argentina	Cactus	Nonfertile		tr
51.	R-7721	NRRL-13927	P. Nelson	USA, MT	Soil	Nonfertile		tr
52.	R-7738	A-27979	P. Nelson	Thailand	Soil	Nonfertile		tr
53.	R-7847	A-27981	P. Nelson	USA, AK	Tundra	Nonfertile		tr
54.	R-7849	A-27982	P. Nelson	USA, AK	Tundra	Nonfertile		tr
55.	R-7850	A-27983	P. Nelson	USA, AK	Tundra	Nonfertile		tr
56.	R-7851	A-27984	P. Nelson	USA, AK	Tundra	Nonfertile		tr
57.	R-7852	A-27967	P. Nelson	USA, AK	Creek bank	Nonfertile		0[j]
58.	R-7853	A-27985	P. Nelson	USA, AK	Creek bank	Nonfertile		tr
59.	R-8135	A-27986	P. Nelson	South Africa	Soil	Nonfertile		tr
60.	R-8177	A-27987	P. Nelson	South Africa	Debris	Nonfertile		tr
61.	R-8178	A-27988	P. Nelson	South Africa	Debris	Nonfertile		tr
62.	R-8179	A-27989	P. Nelson	South Africa	Debris	Nonfertile		tr
63.	R-8182	A-27990	P. Nelson	South Africa	Debris	Nonfertile		11
64.	R-8183	A-27991	P. Nelson	South Africa	Debris	Nonfertile		4
65.	R-8411	A-27992	P. Nelson	South Africa	Soil	Nonfertile		16[j]
66.	R-8429	A-27993	P. Nelson	South Africa	Debris	Nonfertile		2
67.	R-8430	A-27994	P. Nelson	South Africa	Debris	Nonfertile		tr
68.	R-8438	A-27995	P. Nelson	South Africa	Debris	Nonfertile		tr
69.	KF-366	NRRL-13932	J. Chelkowski	Poland	Potato	Nonfertile		0[j]
70.	KF-725	NRRL-13930	J. Chelkowski	Poland	Hops	Nonfertile		0[j]
71.	None	NRRL-13501	H. Abbas	USA, AK	Grass	Nonfertile		18
72.	None	NRRL-13502	H. Abbas	USA, AK	Grass	Nonfertile		3
73.	A-27938[h]	NRRL-13712	A. Murphy	Canada, N. B.	Potato	Nonfertile		0

lial plug excised from a culture growing on V8 juice agar. Radial growth was measured daily on experimental and control plates (DMSO only) for 7 days, or until growth had reached the edge of the plate. Red pigment production by strains was determined following 10–14 days of growth on potato-dextrose agar as previously described (10).

RESULTS

Identification of new fertile strains. As shown in Table 1, eight of the 53 new strains tested formed fertile crosses. Strains R-5920, R-5928, KF-702, KF-710, A-27966, and NRRL-13495 were identified as mating type 1 and strains A-27937 and A-27940 were identified as mating type 2. All eight strains functioned as males; three of these strains, A-27940, KF-702, and KF-710 also produced protoperithecia and are therefore hermaphrodites. The total number of fertile strains in our collection of 73 strains is 27 (Table 1).

Further characterization of selected fertile strains by genetic crosses. Nine mating type 1 strains, R-583, R-2633, R-5390, R-5920, R-5928, R-6112, NRRL-13500, DAOM-192963, and DAOM-192966, were tested as both donors and recipients in crosses with three mating type 2 strains: R-2882, R-5389, and the mating type 2 tester strain R-5455. All crosses were fertile or infertile in accordance with the determined mating type and sex of both parents, with the following exceptions. R-5920 (Mln^+) and R-5928 (Mln^+) failed to yield fertile crosses with the hermaphroditic recipient strain R-2882. The use of DAOM-192966 (Mln^+, Fmn^+) as the female strain and either R-5389 (Mln^+) or R-5455 (Mln^+, Fmn^+) as the male strain did not yield fertile crosses. Similarly, crosses between the protoperithecia-producing strain R-583 (Mln^+, Fmn^+), as the female, and each of the three Mat-2 strains, as the male, were infertile. The reasons for these cases of infertility are unknown.

In selected crosses involving the above nine strains, we also examined the transmission of a gene or genes for red pigmentation (Table 2). These experiments were done to confirm that both parents participated in each cross, as demonstrated by the recovery of either both parental phenotypes and/or recombinant phenotypes among the progeny, and to obtain some information about the number of different genes that confer a red phenotype. All seven crosses between $Red^+ \times Red^-$ strains showed segregation for both phenotypes. In five of these crosses, Red^+:Red^- progeny were recovered in a 1:1 ratio. This suggests that each pair of strains differ at one locus for this trait. The remaining two $Red^+ \times Red^-$ crosses yielded segregation ratios of Red^+:Red^- progeny that were closer to 1:3. This pattern of inheritance is consistent with the two parents in each cross differing at two loci where one of the two loci is segregating as a suppressor of the Red^+ allele at the other locus. As such, the Red^+ parent would be $Red1^n$ (i.e., nonsuppressor of $Red2^+$) $Red2^+$, and the white parent would be $Red1^s$ (i.e., suppressor of $Red2^+$) $Red2^-$; this hypothesis can be tested by additional crosses. Three $Red^+ \times Red^+$ crosses were also done. In one (R-5455 × R-583) all the progeny were red. Because both strains participated in crosses with Red^- strains, the recovery of all red progeny suggests that R-5455 and R-538 are allelic for at least one Red locus. In the remaining two $Red^+ \times Red^+$ crosses, both Red^+:Red^- progeny were recovered. This suggests that more than one locus is responsible for the red phenotypes. However, if two independent loci were segregating for the red phenotype, then a 3:1 ratio of Red^+:Red^- progeny would be expected instead of the observed 1:1 ratio. The 1:1 ratio could occur if two loci were responsible for the red phenotype, and spores that carried the Red^+ allele from one of the loci failed to develop. Again, this possibility could be tested in future studies by tetrad analysis. Despite the unusual segregation of phenotypes in the last two crosses, the above results from all the crosses served to demonstrate that there is more than one locus in *G. pulicaris* strains that determine the red phenotype, and that all the strains participated in the crosses rather than simply inducing each other to form self-fertile perithecia.

The inheritance of mating type was investigated in one cross, 325, by random ascospore analysis and in another cross, 287, by tetrad analysis. In cross 325 (R-6380 [Mat-1, Mln^+, Fmn^+] × R-2882 [Mat-2, Mln^+, Fmn^+]), where R-6380 served as the recipient strain, the mating type of 52 random ascospore progeny was tested by backcrossing each one to both parents. The original parents were used as the recipient strains and the ascospore progeny as the donor strains. Twenty-five (48.1%) progeny formed fertile crosses with R-2882 and, therefore, are Mat-1; 15 (28.8%) progeny formed fertile crosses with R-6380 and are Mat-2; and 12 (23.1%) progeny failed to cross with either parent. This same pattern was observed previously for cross 287 (R-6380 [Mat-1, Mln^+, Fmn^+] × R-5455 [Mat-2, Mln^+, Fmn^+]) (10). Here, we re-examined the inheritance of mating type in cross 287 by tetrad analysis and by making crosses to selected siblings as well as repeated backcrosses to both parents. This approach yielded fertile crosses for all the progeny and a 1:1 segregation ratio for Mat-1:Mat-2 (Table 3). These results confirm that a gene for mating type, *MAT*1, is segregating as two alleles, *MAT*1-1 and *MAT*1-2, in this cross. Segregation data for *Tox*1 in these same tetrads has been previously reported (5); the *Tox*1 gene determines the hydroxylation state of the C-8 carbon in trichothecenes. Analysis of the inheritance of both mating type and C-8 hydroxylation demonstrated that *MAT*1 and *Tox*1 are not closely linked; the *MAT*1-1, *Tox*1$^+$; *MAT*1-1, *Tox*1$^-$; *MAT*1-2, *Tox*1$^+$; *MAT*1-2, *Tox*1$^-$ genotypes occurred as a near 1:1:1:1 ratio of 6:4:4:6 ($x^2 = 0.8$; $P > 0.8$. (Table 3). The segregation pattern of red pigmentation and mating type (Table 3) supports previous random ascospore data, which indicated that the genes for these two traits are also not closely linked (10). In the present study, the *MAT*1-1, Red^+; *MAT*1-1, Red^-; *MAT*1-2; Red^+; *MAT*1-2, Red^- genotypes occurred in a near 1:1:1:1 ratio of 6:4:3:7 ($x^2 = 2.0$; $P > 0.5$).

Search for new genetic markers among the fertile *G. pulicaris* strains. As demonstrated by the foregoing genetic analyses of fertile crosses between naturally occurring strains, not only is the known number of allelic differences among these strains limited, but those that are available (such as mating type and C-8 hydroxylation of trichothecenes) are extremely time-con-

TABLE 2. Inheritance of red pigmentation in selected crosses

Cross no.	Parental strains recipient × donor	Random ascospore progeny			Probable ratio	Chi-square test
	Mat-2 × Mat-1	Number picked	Percent germinated	Red^+:Red^-		
188	R-2882 (Red^-) × R-583 (Red^+)	50	66	16:17	1:1	$x^2 = 0.030$; $P > 0.9$
297	R-5455 (Red^+) × R-583 (Red^+)	48	92	44:0	1:0	
279	R-5455 (Red^+) × R-5390 (Red^-)	54	100	23:31	1:1	$x^2 = 1.185$; $P > 0.2$
284	R-5455 (Red^+) × R-6211 (Red^-)	54	94	28:23	1:1	$x^2 = 0.490$; $P > 0.4$
313	R-5455 (Red^+) × R-2633 (Red^-)	54	87	17:30	1:3	$x^2 = 3.125$; $P > 0.06$
712	R-5455 (Red^+) × NRRL-13500 (Red^-)	54	89	21:27	1:1	$x^2 = 0.750$; $P > 0.35$
715	R-5455 (Red^+) × DAOM-192963 (Red^-)	54	74	18:22	1:1	$x^2 = 0.400$; $P > 0.5$
716	R-5455 (Red^+) × DAOM-192966 (Red^-)	54	83	14:31	1:3	$x^2 = 0.939$; $P > 0.3$
1021	R-5455 (Red^+) × R-5920 (Red^+)	54	93	25:25	1:1	$x^2 = 0.000$; $P > 1.0$
1036	R-5455 (Red^+) × R-5928 (Red^+)	54	93	25:25	1:1	$x^2 = 0.000$; $P > 1.0$

suming to score. In the hope of identifying more readily scorable genetic differences, we tested the sensitivity of some of these strains to a variety of compounds (Table 4). Resistance to benzimidazoles has been reported in *G. pulicaris* (29), however, all isolates studied here were very sensitive to both benomyl and thiobendazole. In addition, most of the *G. pulicaris* isolates tested were very similar in sensitivity to a wide variety of antibiotics and fungicides, including aminoglycosides, betalactams, pyrimidines, triazoles, and imidazoles. Although this survey did indicate some potentially useful antibiotic resistance markers, preliminary genetic analysis demonstrated that resistance was inherited polygenically. Thus, no useful genetic markers were found.

Strains of *G. pulicaris* are also known to show differential tolerance to the plant phytoalexins, rishitin, lubimin, and xanthotoxin (11,12,14,17). Earlier studies had revealed that of the genetically fertile strains examined at that time, one, R-7843, was tolerant of rishitin (11) and one, R-5920, was tolerant of xanthotoxin (14). Twelve of the genetically fertile strains not previously examined for tolerance to rishitin, lubimin, and xanthotoxin were tested here. No new sensitive strains were found; thus, all genetically fertile strains of *G. pulicaris* are tolerant of rishitin (except R-7843), lubimin, and xanthotoxin (except R-5920).

Search for new mating populations. The failure of 46 of the 73 strains in Table 1 to form fertile crosses with either R-6380 (*MAT*1-1) or R-5455 (*MAT*1-2) could be indicative of the existence of one or more additional mating populations. This possibility was explored by selecting 31 of the infertile strains for further analysis. In the first experiment, 15 infertile strains (R-110, R-2155, R-5185, R-5214, R-5344, R-5683, R-5684, R-5690, R-5749, R-5753, R-5867, R-7570, R-7715, NRRL-13501, NRRL-13502) were crossed in all pairwise combinations to each other (420 cross combinations) as well as to 12 fertile strains (R-583, R-2633, R-2882, R-5389, R-5390, R-5920, R-5928, R-6112, R-6354, DAOM-192963, DAOM-192966, NRRL-13500) (360 cross combinations). In the second experiment, 25 recipient strains (R-2155, R-3084, R-3245, R-4263, R-4268, R-4272, R-4273, R-5344, R-5683, R-5684, R-5690, R-5749, R-5867, R-7570, R-7847, R-7851, R-7852, R-8135, R-8179, R-8183, R-8411, R-8429, R-8430, R-8182, NRRL-13502), representative of three major groups of infertile strains (based on their geographical origin and habitat), were crossed to seven donor strains (R-3084, R-3245, R-5690, R-7570, R-7847, R-7852, and R-8430) to yield 193 pairwise combinations. None of the above crosses were fertile; consequently, these experiments did not reveal the existence of any other mating populations within *F. sambucinum*.

Trichothecene production. The ability of the *F. sambucinum* strains to produce diacetoxyscirpenol in liquid shake cultures is recorded in Table 1, which includes data for the 53 new strains as well as for the 20 previously characterized strains in our collection. There was little difference in growth as measured by dry weight between the high to low trichothecene producers (34 strains; dry weight = 6.2 mg/ml ± 3.3) and the very low (<1 μl/ml) to possible nonproducers (36 strains; dry weight = 6.7 mg/ml ± 2.6). All of the fertile strains we have identified make detectable amounts of DAS and therefore are capable of making trichothecene toxins. In fact, of the 73 strains tested, only five may be unable to produce trichothecene toxins. Because all five strains demonstrated good growth as measured by dry weight, the inability to detect trichothecenes is not due to an inability of the strains to grow in liquid shake culture in YEPD-5G medium. However, caution must be used in designating a *Fusarium* strain as toxin-minus based on negative results obtained under only one set of growth conditions.

TABLE 3. Segregation of mating type in cross 287 (R-6380, Mat-1 × R-5455, Mat-2)

Ascus no.	Ascospore[a] nos.	Mating type	*Tox*1[b]	Red[b]
1	1,4, and 2	1	+	+
	3,5, and 7,8	2	−	−
3	1,6	1	−	+
	3	1	−	−
	2,5	2	+	−
	7,4	2	+	−
5	1,5, and 3,4	1	+	−
	2,8, and 6,7	2	−	+
11	5,6	1	+	+
	7,8	1	−	−
	1,4	2	−	+
	2,3	2	+	−
15	3,4	1	+	+
	7,8	1	−	+
	1,6	2	+	−
	2,5	2	−	−

[a] Spore pairs previously identified as twins based on trichothecene levels, *Tox*1 genotype, and red phenotype.
[b] Previously reported data (5).

DISCUSSION

In this study, we examined trichothecene production in liquid shake cultures and determined the sexual compatibility of 53 previously uncharacterized *F. sambucinum* strains. The identification of eight new fertile strains (R-5920, R-5928, KF-702, KF-710, A-27966, NRRL-13495, A-27937, and A-27940) from this group brings the total number of mating-competent strains in our collection of 73 to 27. Thus, approximately one-third of the natural isolates we have tested can form the *G. pulicaris* teleomorph under laboratory conditions. The majority (~70%) of these fertile strains are mating type 1. R-583, one of the strains we identified as Mat-1, is strain SA-177 from Gordon's collection. Because Gordon identified SA-177 as mt-a (G. Neish, *personal communication*), we can compare our results to previous mating-type studies that referred to the two mating types as mt-a and mt-A. Gordon also reported that, of the mating-competent strains he examined, 66% were Mat-1 (mt-a) and 34% were Mat-2 (mt-A) (19).

Geographic distribution of mating type. Gordon isolated Mat-1 strains from across Canada (British Columbia, Alberta, Sas-

TABLE 4. Sensitivity of selected strains of *Fusarium sambucinum* to various antibiotics and fungicides[a]

Compounds tested	Concentration	Sensitivity of strains: Percentage of inhibition of growth
Sulpha drugs		
5-Methoxysulfadiazine	1.0 mM	0–25
Sulfadiazine	1.0 mM	0–56
Sulfamerazine	1.0 mM	0–25
Sulfamethozine	1.0 mM	0–25
Sulfaminouracil	1.0 mM	0–8
Sulfisomidine	1.0 mM	0–11
Sulfisoxazone	1.0 mM	0–14
Cyclopeptides:		
Polymyxin	500 μg/ml	46–89
Enniatin B	50 μg/ml	45–60
Steroid glycoalkaloid:		
Tomatidine	100 μg/ml	8–44
Sterol biosynthesis inhibitor		
Triadimefon	50 μM	17–72 E.D. 50[e]
Benzimidazoles:		
Benomyl	0,1,5,10,50 μM	2.6–7.8 μM
Thiabendazole	0,1,5,10 μM	5.2–20 μM

[a] Strains tested: R-583, R-2633, R-2882, R-5389[b], R-5390, R-5455[b], R-5920[c,d], R-5928[c,d], R-6112[d], R-6354[c,d], R-6380[b], NRRL-13500[c], DAOM-192963[c], DAOM-192966[c]
[b] Three strains tested with amcymidol, imazalil, triforine, and RH 53866; all were equally sensitive to each compound.
[c] Strains not tested with enniatin B.
[d] Strains not tested with tomatidine.
[e] E.D. 50 = concentration at which there was 50% inhibition of the rate of radial growth, measured daily for 7 days.

katchewan, Manitoba, New Brunswick, and Prince Edward Island) and both mating types from Manitoba, Prince Edward Island, and England (19). In our studies, both mating types were present in strains from New Brunswick (Canada), the north central United States (Wisconsin and Minnesota), Iran, and Australia. Although it is apparently only rarely found, the teleomorph also occurs in nature and several researchers have reported its isolation (7). Based on these studies, there is no obvious differential geographic distribution of mating type in *G. pulicaris* other than the more common isolation of Mat-1 as noted above.

As previously reported for this species (8,19), we have observed only heterothallism and a single mating population. None of the isolates examined formed the teleomorph alone, and we found no evidence for additional mating groups, although such groups have been reported for other *Fusarium* species (8,23,25,28).

Distribution of sex. In both mating types, slightly more than half of the strains were unisexual males (10 of the 19 Mat-1 strains and five of the eight Mat-2 strains). All of the remaining strains were hermaphrodites. Although fertile unisexual females occur in other *Fusarium* species (32), none were observed in the present study, nor have they been previously reported for *G. pulicaris*.

Inheritance of mating type. Early reports indicated a 1:1 Mendelian segregation for alleles at a single gene locus controlling mating type in *G. pulicaris* (16,20). We have also previously observed a simple 1:1 segregation of mating type (10,11). However, in both the present study and a previous study, a portion of the random ascospore progeny from three different crosses failed to form fertile backcrosses and a 1:1 segregation ratio for mating type had to be inferred. In one case, (cross 287: R-6380 × R-5455), ~50% of the progeny were Mat-1, ~25% were Mat-2, and ~25% were infertile, but presumably Mat-2 (10). This cross was reexamined in the present study by tetrad analysis. Fertile crosses were eventually obtained for all the progeny and the results clearly demonstrated 1:1 segregation of mating type. These data also raise some cautions in assessing strains as infertile; strains determined to be infertile could prove to be fertile in future crosses with other strains or under other conditions. Finally, it should be noted that the failure of some crosses involving field strains of filamentous fungi is not atypical as they have not been bred for increased fertility. This problem has been addressed, in part, for *G. pulicaris* by the development of a highly fertile, near isogenic set of strains (3).

Trichothecene production, fertility, and strain origin. All the fertile strains of *F. sambucinum* examined made trichothecenes, however, they did vary in the type and amount they produced. As reported earlier, some strains (designated $C8^+$) were able to hydroxylate the C-8 position on the trichothecene nucleus and others (designated $C8^-$) were not (5). $C8^+$ and $C8^-$ strains were present in both mating types, and there seemed to be little difference in the distribution of $C8^+$ and $C8^-$ strains between the two mating types (data from present study not shown). Both mating types also contained strains that have the ability to produce large quantities (>200 μg/ml) of DAS (Table 2). However, if each mating type is considered overall (i.e., after excluding the one exceptionally high >200 μg/ml producer in each case), then the Mat-1 strains produced higher titers of DAS in liquid culture than did the Mat-2 strains (58 ± 45 μg/ml verses 19 ± 29 μg/ml; $P < 0.05$ by Student's t test). Although this difference in DAS production levels is significant, the current sample size of Mat-2 field strains is small, and a definitive conclusion on this point must await additional data.

In the present study, a high correlation was observed between trichothecene production and fertility. The majority (25 of 27) of the fertile strains were good (1–20 μg/ml) to very good (20–400 μg/ml) trichothecene producers whereas the majority (35 of 46) of the nonfertile strains were poor (<1 μg/ml) trichothecene producers. In fact, only one very good trichothecene producer (R-7570) was nonfertile.

The correlation between trichothecene production and fertility was further correlated with the isolation of strains from diseased plants other than grass (Fig. 1). Of the 27 fertile strains, 22 were isolated from diseased plants, three were of unknown origin, and only two (R-5928 and R-6112) were isolated from soil. Of the 46 nonfertile strains, 26 were isolated from soil or debris, 12 were isolated from grass, two were of unknown origin, and only six were isolated from diseased plants. Among the six nonfertile strains isolated from diseased plants, three (KF-366, KF-725, and A-27938) did not make detectable trichothecenes, one (R-7715) made only trace levels of DAS, and one (R-5344) made low levels of DAS. The sixth strain, R-110, made protoperithecia, and therefore showed some signs of fertility. Thus, out of the 73 strains examined, all 27 of the fertile strains made trichothecenes and only two of these strains were clearly documented to be isolated from soil. Conversely, only two strains that were both isolated from a diseased plant and made some DAS showed no sign of forming a fertile cross.

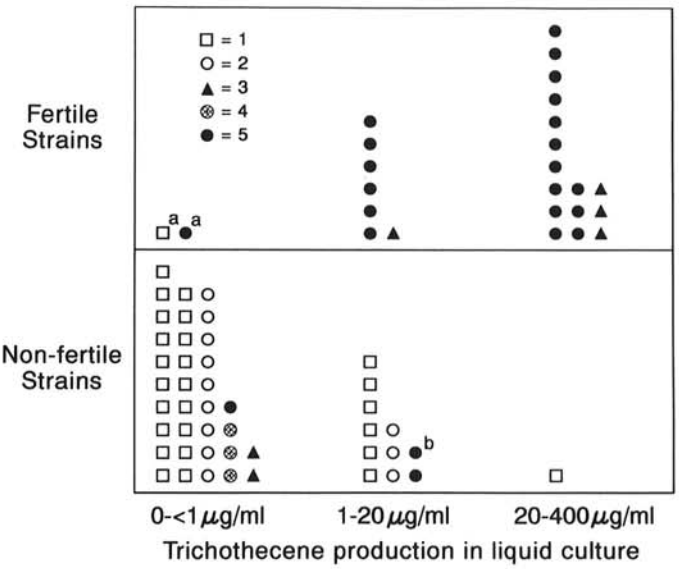

Fig. 1. Correlation between fertility, origin, and trichothecene production in liquid shake culture for *Fusarium sambucinum* strains used in this study. The symbols in this figure correspond to the following numbers as indicated: 1, denotes a strain that was not isolated from a diseased plant; 2, denotes a strain isolated from grass; 3, denotes a strain of unknown origin; 4, denotes a strain that was isolated from a diseased plant but which made no detectable trichothecenes under the growth conditions employed in this study; and 5, denotes a strain that was isolated from a plant and was able to make trichothecenes. a, These two strains (R-5920 and R-5928) are very difficult to cross. In numerous attempts they have crossed only twice with one strain, R-5455. b, This represents strain R-110, which produces protoperithecia.

The strong correlation between toxin production, fertility, and origin of the strain raises the possibility that trichothecene production, pathogenicity, and/or fertility may be interdependent traits. A common denominator could be trichothecene production. Previous work with T-2 toxin-minus mutants indicated that trichothecene production was required for the pathogenicity of *F. sporotrichioides* on parsnips (15). This same requirement could hold true for *G. pulicaris* on its host plants. Trichothecene production could also be involved with fertility. Other fungal and plant secondary metabolites have been implicated or shown to be fertility factors (18,33). We are currently attempting to isolate *G. pulicaris* mutants specifically blocked in trichothecene biosynthesis to further examine these possibilities. If trichothecene toxins do play an essential role in pathogenicity and/or fertility, then true trichothecene toxin-minus strains of *G. pulicaris* would be nonpathogenic and/or sterile.

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